

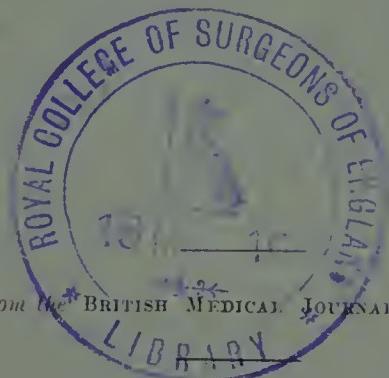
OPSONINS AND THEIR UTILITY IN PRACTICAL MEDICINE.

AN ADDRESS READ BEFORE THE
MEETING OF THE TUNBRIDGE WELLS AND NEIGHBOURING DIVISIONS
OF THE BRITISH MEDICAL ASSOCIATION.

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ON OPSONINS AND THEIR UTILITY IN PRACTICAL MEDICINE.

I do not wish it to be supposed that what I have here to say about opsonins and the question of vaccine treatment of certain diseases is any original work of my own; the credit of the work belongs to Koch, Behring, Wright, Bulloch, Eyre, Louisson, and a host of other workers. It is to Sir A. E. Wright that our present knowledge of opsonins is mainly due.

WHAT OPSONINS ARE.

The white corpuscles of the blood can be kept alive outside the body; experiments can therefore be made with living leucocytes in test tubes and so on. Under the microscope the great Professor Metchnikoff watched such leucocytes engulfing or swallowing up bacteria by a process which we all know by the name "phagocytosis." Phagocytosis is at the bottom of all estimations of the opsonic index.

Let me confine myself for the present to the phagocytosis of tubercle bacilli. If living leucocytes in a test tube be incubated at body temperature along with a number of tubercle bacilli obtained from a cultivation of these organisms, a film can be made of the leucocytes upon a microscopic slide, stained appropriately by the Ziehl-Nielsen method for tubercle bacilli, and examined under a microscope with a $\frac{1}{2}$ in. oil-immersion lens. It will be found that many of the tubercle bacilli are now inside the leucocytes, having been, so to speak, eaten by them. The number thus eaten up by 100 leucocytes can be counted. We shall all agree that the larger the number of tubercle bacilli taken up by 100 leucocytes in this way in a given time, the greater is the phagocytic power of those leucocytes for the tubercle bacilli in question. For instance, granted that the same cultivation of tubercle bacilli be used in each case, and that the time and temperature of incubation be the same, if it be found that one set of leucocytes engulf 100 tubercle bacilli per 100 leucocytes, and another set engulf 200 tubercle

bacilli per 100 leucocytes, then the phagocytic power in the latter case is twice that in the former.

The next question is, what are the factors which cause variations in the phagocytic power of leucocytes? There are, of course, many factors—such as the temperature of the incubator, the freshness of the leucocytes, the virulence of the tubercle bacilli, and so on. We do not need to consider all these.

I.

If I were to obtain one set of quite fresh leucocytes from my own blood, and another set of quite fresh leucocytes from a phthisical patient's blood, and some blood serum from a healthy third person, and were then to prepare two test tubes, putting into each the same amount of the same culture of tubercle bacilli, and the same quantities of the serum, and then added my leucocytes to the one, and the phthisical patient's leucocytes to the other, and if after incubating the two for the same length of time I prepared films, and counted the tubercle bacilli taken up by 100 of each of our leucocytes, I should find that the phthisical patient's leucocytes and my leucocytes would have taken up almost exactly equal numbers of bacilli—that is to say, leucocytes from different human sources, mixed with the same blood serum, have approximately equal phagocytic powers for a given microbe.

II.

The next step is almost the converse of the preceding. If I were to prepare some quite fresh leucocytes from the blood of the healthy third person, and some serum from my own blood and some serum from the phthisical patient's blood, and were then to prepare two test tubes, putting into each the same amount of the same culture of tubercle bacilli, and the same quantity of the third person's leucocytes to each, and then added my serum to the one and the phthisical patient's serum to the other; and if, after incubating the two for the same length of time, I should again prepare films, and count the tubercle bacilli taken up by 100 of the third person's leucocytes in each tube, then if the phthisical patient were fairly ill, I should find that though the leucocytes were in each case from the same healthy source, they would have taken up far more tubercle bacilli when mixed with my serum than when mixed with the phthisical patient's serum.

It will be seen from the two series of experiments that the phagocytic power of human leucocytes depends very little upon the leucocytes themselves, but very much upon the blood serum in which those leucocytes are. The something in the serum which influences the phagocytic power of the leucocytes has not been isolated, and how it influences phagocytosis is uncertain. It is thought that the serum contains substances which act upon the bacteria, rendering them more easily eaten by the leucocytes. These substances are the opsonins, a name derived from Greek words which mean "prepared for being eaten."

THE OPSONIC INDEX.

My next duty is to explain what is meant by the opsonic index. Indirectly I have already done so, but I will describe an imaginary case. Suppose I wished to estimate my own opsonic index to tubercle bacilli. I should prepare blood serum from myself, obtain some blood serum from another person known to be healthy, some fresh leucocytes no matter from whom, and a culture of tubercle bacilli. I should prepare two test tubes—

Putting into the first :

Leucocytes,
Some of the tubercle bacilli,
And my serum.

And into the second :

An equal quantity of the leucocytes,
An equal quantity of the tubercle bacilli,
The serum of a healthy person.

I should then incubate both test tubes at the same temperature, preferably body heat, and each for the same length of time. It does not much matter how long the incubation is, provided it be the same for my test tube as for the control. I should then make films from each test tube, and count the bacteria engulfed by an equal number of leucocytes in each. If in what I call "my" tube 100 leucocytes had taken up 150 tubercle bacilli, and in the control tube 100 leucocytes had taken up, say, 300, my opsonic index to tubercle bacilli would be $\frac{15}{300}$, or 0.5. The opsonic index is, in other words, the ratio of the bacilli engulfed by leucocytes incubated in the patient's serum to those engulfed by an equal number of leucocytes incubated under similar circumstances in healthy serum.

The estimation of an opsonic index takes time, three to four hours even in a bacteriological laboratory; but when many can be carried on simultaneously it does not take a great deal longer to do several. I will explain all that is necessary to be done by the medical man himself. He needs a small glass bulb, with open capillary ends. A simple Widal tube is as good as any, and the method of getting the small quantity of blood required is precisely similar to that we are constantly using for the Widal test in typhoid fever. The lobule of the ear is reddened by rapid friction; it is then sharply pricked with a surgical needle. The capillary end of the Widal tube is held to the drop of blood that comes, and by simple gravity and capillary attraction, without suction, the tube is filled at the one end, care being taken not to let the blood run down to the other. Both ends are sealed off in a flame, the clear end first, and the tube left horizontal for about twenty minutes. The blood will then have completely clotted, and serum from the clot, almost free from corpuscles, will run down into the free end when the tube is inverted. The tube is put back into its case, placed in an envelope, and posted to the laboratory. The medical man has finished his share of the work. The next day or the day after the report comes back saying that the opsonic index to such-and-such an organism is so-and-so.

VARIOUS OPSONINS.

Each blood serum contains many different opsonins—one variety for each sort of microbe. For example, the opsonic index of a given serum may be estimated against tubercle bacilli, staphylococci, gonococci, streptococci, and so on, by incubation with these different organisms respectively, and it does not follow that the opsonic index of a given serum will be high to staphylococci because it happens to be high to tubercle bacilli, and so on. It is necessary to specify the particular organism against which the opsonic index is to be estimated in each individual case.

DIAGNOSTIC VALUE OF OPSONIC INDEX (1).

In the case of tubercle bacilli, the opsonic indices of a series of healthy individuals may vary from 0.8 to 1.2, or thereabouts, but in tuberculous patients the index may be as low as 0.3 or as high as 1.8, or even higher. The estimation, therefore, may be of considerable diagnostic value in cases where there is doubt as to whether the lesion is tuberculous or not. If the index is below 0.7 or above 1.3, the argument will be in favour of tubercle, the resisting power of the patient being low in the first case, high in the second.

THE EFFECTS OF VACCINES UPON THE OPSONIC INDEX AND THE PRINCIPLES OF VACCINE TREATMENT IN DISEASE.

It is known that living organisms, during their growth, produce waste products inimical to the organisms themselves. Yeast is one of the simplest examples of this. Yeast lives upon grape sugar, decomposing it and producing alcohol; alcohol is inimical to yeast; when the percentage of alcohol in a fermenting sugar solution reaches a certain point, further fermentation ceases because the yeast can no longer grow. Microbes in culture media produce antibodies in a similar way.

Ever since Koch's earlier discoveries there have been repeated attempts to turn these facts to therapeutic account. Koch himself hoped to cure phthisis and other tuberculous lesions by injecting patients with extracts of tubercle bacilli. The results were bad, death often being even accelerated. The method of treatment fell into disrepute. It is now reviving, and with good results.

The amount of tuberculin injected by Koch was far too large, and there was no method of checking its effects. It is owing to Wright's investigations upon opsonic indices that we have now learned what quantity of tuberculin should be given, when it should be given, and when the dose should be repeated.

Vaccine treatment, with or without estimations of the opsonic indices, is not a means of working absolute miracles, and it must not be supposed that all other forms of treatment at once become unnecessary. It is a method of very real benefit to many patients, but it is to be used in addition to, not in place of, other well-recognized procedures.

DIAGNOSTIC VALUE OF OPSONIC INDEX (2).

It has been found that, after injecting $\frac{1}{500}$ mg. of tuberculin R. into a healthy man, the opsonic index falls slightly for about two days, then rises to slightly above normal, and then returns to what it was originally. A similar injection into a tuberculous patient is followed by a considerable fall in the opsonic index, the latter remaining below what it originally was for a week or more, by which time it has begun to rise again, until in between two and three weeks it has risen above what it was before. The initial fall after the injection is called the negative phase. This negative phase is quite short in healthy people, long in tuberculous subjects, so that we have here an additional means of diagnosis. Formerly, when tuberculin was used as a diagnostic test, it was given in large doses, as it is still in cattle; the test was not positive unless there was both local reddening and pain, and a general reaction with increased pyrexia and illness. With the dose now used, $\frac{1}{500}$ mg., there is no such gross reaction; the patient does not feel the least ill effects, and need not interrupt his work; but, if the opsonic index be taken every other day, and the negative phase extends to a week or more, the suspected lesion is most probably tuberculous.

With larger doses of tuberculin the negative phase becomes more marked; a second injection during the negative phase of the first increases the fall, and postpones the subsequent rise. This explains the bad results originally obtained by Koch and others. Much too large a dose of tuberculin was given to start with; the doses were repeated at much too short intervals; the patient's opsonic index was lowered and kept low, diminishing his resisting power and enabling the tubercle bacilli to make fresh inroads, and perhaps kill him from general tuberculosis.

In treating a patient now, the opsonic index of his serum to tubercle bacilli must first be taken. If it is already high, tuberculin treatment will probably effect little, because the patient's tissues are already reacting well, and can probably not be made to react much better by tuberculin. If, on the other hand, the opsonic index be low, or at any rate not high, a dose of $\frac{1}{500}$ mg. of tuberculin R., obtained in a tiny glass bulb ready sterilized, is taken up in a hypodermic syringe and injected under the skin of the arm or elsewhere, just as in giving a hypodermic injection of morphine. Aseptic precautions in regard to the syringe and the skin are, of course, observed. The opsonic index is estimated at intervals—say upon the third, seventh, fourteenth, and seventeenth days; if it shows no appreciable change, either the patient is not tuberculous or the dose requires to be increased. In most tuberculous cases the $\frac{1}{500}$ mg. will be sufficient; the negative phase will be over within a fortnight, and in about three weeks from the first injection the positive phase will be well marked. There is no need then to increase the dose, but at the height

of the positive phase the next injection of $\frac{1}{100}$ mg. may be given. The opsonic indices need not necessarily be taken any more, though it might be safer to take them. The great probability is that the third injection will be required at the same time after the second that the second was after the first; so that, having used the opsonic indices to find out how the patient reacts and what time should elapse between the doses in his particular case, the further treatment by tuberculin can be carried on by injections at regular intervals without any necessity for the expense of further opsonic index estimations.

The value of this method of treatment has been proved in many cases of local tuberculous affections—particularly lupus, ulcers of the skin, and tuberculosis of the bladder. Cure does not come about as by a miracle; the lupus cases need light or other form of treatment as well as tuberculin; the tuberculous bladder cases require urotropin and so on as well as tuberculin; but if you could see the relief that has been given in some of the cases by the addition of tuberculin injections to other remedies I am sure you would give them a trial.

STAPHYLOCOCCAL VACCINES.

Vaccines have been made from cultivations of staphylococci, gonococci, and so on, and used as injections similar to those of tuberculin, controlled, both as to dose and as to interval between doses, by opsonic index estimations precisely as above. I have had little or no experience of this treatment in gonococcal, streptococcal, or pneumococcal affections, but I have used it many times for staphylococcal troubles.

Acne Vulgaris.

We all know how difficult it is sometimes even to relieve acne; any additional means of combating this common complaint must be welcome. Though the vaccine treatment is not a panacea, I have found it of the greatest service in making other methods of treatment successful. I have seen complexions enormously improved by it after years of disfigurement from constant blains and boils. The cases treated so far have naturally been the worst, but I believe it possible to save complexions by adopting the vaccine treatment earlier.

The organism is the staphylococcus, but there seem to be different brands in different cases, or possibly different mixtures of the three varieties—*aureus*, *albus*, and *citreus*. The results obtained with vaccines from one culture of staphylococci may be very different from the results obtained from another. It is necessary, therefore, to make the vaccine in each case from the patient's own microbes. All one has to do is to break the top of a pustule in the absence of all antiseptics; collect a little of the pus on a swab; smear it upon the surface of the culture medium in a test tube, which can be obtained from the laboratory; replace the wool stopper; put it into a metal case, and post it back to the laboratory. There they incubate the

culture, make an emulsion of it, calculate the number of organisms present, sterilize it, filter it, measure off such quantities as contain the products of 250,000,000 staphylococci, seal each quantity in a glass bulb, sterilize the bulbs once more, and post them ready for use. The dose is the content of one bulb. It is injected by means of a hypodermic syringe. No feeling of illness results, and the patient's daily work is not interfered with. For one or two days there is no result; the patient then experiences a feeling of being better, the spots and blains grow less, until, about the seventeenth day or a little earlier, the patient begins to feel he is going back again. This is the time for a second dose.

The effect does not last indefinitely; after half a dozen doses at similar intervals the pimples and boils can usually be kept under with very little trouble by means of the usual local applications; but in my experience it is necessary to go on with the injections at intervals of about three weeks for a long time. In the case of ladies especially this is well worth while, in order to preserve the complexion until such time as the acne will spontaneously cease of itself.

Opsonic Index Estimations not necessary in the Vaccine Treatment of Acne.

I have made no mention of the opsonic index in connexion with this treatment by staphylococcal vaccine. The reason is that I do not think estimations of the opsonic index necessary, unless, perhaps, at the very beginning of the treatment. The opsonic indices have been worked out in so many cases of acne treated in this way, and the results have been so constant that their repetition in each individual case is not needed. The negative phase follows the injection just as it does after tuberculin; the positive phase follows the negative, and reaches its maximum very constantly on or about the seventeenth day, so that we know exactly when the second injection should be given, and so on. We could not have learned this without the assistance of opsonic indices in the first instance; but now that we do know it, opsonic indices in cases of acne are no longer necessary in practical medicine. In this respect acne is very different to tuberculosis; in the latter, the best time for the second dose of tuberculin varies greatly in different patients, and can only be determined by taking the opsonic index at intervals after the first dose.

Other Vaccines.

It is not improbable that similar methods of vaccine treatment will be devised against gonococcal and other local microbial infections, now that dosage and effects can be gauged by so definite a means as the opsonic index. Experiments have already been made in this direction, but I am not yet in a position to give you an account of the results.

ILLUSTRATIVE CASES.

I will now give some definite instances in which the tuberculin and staphylococceine injections have done good.¹

Tuberculous Caries and Dactylitis.

A small girl was sent by Dr. Reynolds of Wycombe, for tubereulin treatment at Guy's Hospital. She had tuberculous dactylitis of the right thumb and tuberculous caries of one os calcis, both accompanied by discharging sinuses and much swelling. Local and constitutional treatment had been tried in vain for many months; the trouble persisted and was increasing in severity. The opsonic index to tubercle was found to be low. Tuberculin R. $\frac{1}{5000}$ mg. was injected. The opsonic indices showed that between three and four weeks was the proper interval between injections in her case. There was little change in the lesions until after the second dose. Improvement then set in rapidly. After the third dose the swelling of the foot had entirely subsided, and the sinus over the os calcis was healed; the dactylitis was very much better. After the fourth dose the sinus over the thumb had ceased to discharge; there was still some local swelling, but the condition, previously obstinate and persisting, has become practically well in four months.

Acne of Face and Back.

Two students at Guy's Hospital, each of whom had suffered for many years from severe and disfiguring acne, were treated by staphylococceine injections in the manner I have described above. The opsonic index in one case rose from 0.8 to 1.8 after the first injection, and after three injections he states: "My face had by then almost cleared up, and my back was greatly improved; also some hard, indurated glands in my axillæ were very much smaller." The other student experienced even greater relief, and thereafter found it much more easy to keep the boils in check.

Multiple Staphylococcal Abscesses.

An infant at Guy's Hospital, under the care of Mr. F. J. Steward, was suffering from multiple subcutaneous abscesses which very nearly killed it. Altogether seventy-five abscesses developed, and others threatened. As fast as some were opened others appeared. The case seemed hopeless. It was found that the abscesses were staphylococcal. A vaccine was prepared and injected at intervals, with immediate cessation of the formation of fresh abscesses, followed by rapid and complete recovery.

I might multiply these examples indefinitely, but I think the above are sufficient to indicate the benefits to be obtained from careful vaccine treatment, particularly in cases of local tuberculous mischief, and in cases of staphylococcal troubles such as acne, boils, and abscesses.

It will be asked whether tuberculin is of any use in the treatment of phthisis. I cannot yet answer definitely. I have not yet tried it myself, but if controlled by opsonic index estimations I think it is worth trying in some cases, in addition to the recognized sanatorium and other hygienic measures.

It is particularly in the localized tuberculous diseases that it does good, effecting great relief or even cure in the case of tuberculous skin troubles and bladder affections.

One of the surgeons at Guy's Hospital expresses the view that many tuberculous affections which are at present treated mainly by surgeons will in the near future be treated by physicians, and cured by tuberculin without operation. Whether this is to be so or not it is premature to say. Tuberculin and staphylococcin raise the opsonic index for a time only. When the injections cease the opsonic index will in time fall below par again very likely, so that the patient may become again liable to invasion by the microbes. He is, however, no more liable to their attacks than he was before, and if a lesion can be cured by the vaccines once, a relapse or a recurrence can be cured by them again. It is unwise to stop the injections too soon; and it is unwise to depend upon the injections alone, to the neglect of hygienic, medicinal, surgical, and other general measures.

Hitherto most of the cases treated have been very severely ill; vaccine treatment has often been adopted only when surgical and all other measures have failed, for example, in cases of tuberculous ulcers, cystitis, lupus, acne, and so on. Seeing that, in spite of this, Wright is able to say that "it is not a question of the achievement of success in a certain percentage of cases only; up to the present it has been a question of uniform success," I think it will be agreed that this additional method of antimicrobial treatment well deserves to be tried early, and not merely as a last resort.

REFERENCE.

¹ I might mention that there is a growing literature upon the subject, and I would refer to papers by Wright, Bulloch, and others in the *Royal Medical and Chirurgical Society's Transactions* for 1906; to the *Practitioner* for July, 1906; to the *Guy's Hospital Reports* for 1906; and to the *Lancet* for October 27th, 1906, which contains a most interesting paper by Drs. Turton and Parkin, with detailed accounts of 34 cases.

